

RC

268.5

U55

no. 186 National Cancer Institute

1979 **CARCINOGENESIS**

Technical Report Series

No. 186

1979

BIOASSAY OF 4,4'-METHYLENEBIS- (N,N-DIMETHYL)BENZENAMINE FOR POSSIBLE CARCINOGENICITY

CAS. No. 101-61-1

NCI-CG-TR-186

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health



*United States National Cancer Institute,
Carcinogenesis technical report.*

BIOASSAY OF
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

US. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

NIH Publication No. 79-1742

REPORT ON THE BIOASSAY OF
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 4,4'-methylenebis(N,N-dimethyl)benzenamine conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 4,4'-methylenebis(N,N-dimethyl)benzenamine was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly.

Histopathologic examinations were performed at Litton Bionetics, Inc. (4). The rat pathology narrative was written by Dr. A. S. Krishna Murthy (4), the mouse pathology narrative was written by Dr. S. Wyand (4), and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. R. M. Helfand (8) and Dr. J. P. Dirkse, III (9) using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (10).

This report was prepared at METREK, a Division of The MITRE Corporation (8) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (8), task leader Ms. P. Walker (8), senior biologist Mr. M. Morse (8), biochemist Mr. S. C. Drill (8), chemist Dr. N. Zimmerman (8), and technical editor Ms. P. A. Miller (8). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. A. R. Patel (1), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

-
1. Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.
 2. Now with the U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, D.C.
 3. Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammon House Road, Valhalla, New York.
 4. Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland.
 5. Now with Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia.
 6. Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.
 7. EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.
 8. The MITRE Corporation, METREK Division, 1820 Dolley Madison Boulevard, McLean, Virginia.

9. Consultant to The MITRE Corporation, currently a professor in the Department of Statistics at The George Washington University, 2100 Eye Street, N.W., Washington, D.C.
10. Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

SUMMARY

A bioassay for the possible carcinogenicity of 4,4'-methylenebis-(N,N-dimethyl)benzenamine was conducted using Fischer 344 rats and B6C3F1 mice. 4,4'-Methylenebis(N,N-dimethyl)benzenamine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine were, respectively, 750 and 375 ppm for rats and 2500 and 1250 ppm for mice. The compound was administered for 59 weeks to rats and for 78 weeks to mice. The period of compound administration was followed by an observation period of 45 weeks for rats and 13 weeks for mice.

There were no significant positive associations between the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered and mortality among rats or mice of either sex. Adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. There was slight dose-related mean body weight depression among female rats, the mean body weight of high dose male rats was slightly less than that for controls, and the mean body weights of dosed mice were significantly lower than their controls, indicating that the concentrations of 4,4'-methylenebis-(N,N-dimethyl)benzenamine administered to these animals in this bioassay may have approximated the maximum tolerated concentrations.

For both male and female rats, there was a significant positive association between the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered and the incidences of follicular-cell carcinomas of the thyroid (i.e., 1/18, 4/50, and 21/46 in the control, low dose, and high dose males, respectively; and 0/20, 3/46, and 23/45 in the control, low dose, and high dose females, respectively). The high dose to control Fisher exact comparisons were also significant for each sex.

Liver neoplasms were observed among male and female mice. There were elevated incidences of hepatocellular adenomas in dosed mice when compared to controls (i.e., 2/20, 3/50, and 16/48 in control, low dose, and high dose males, respectively; and 1/19, 18/49, and 22/48 in control, low dose, and high dose females). The incidences of hepatocellular carcinomas in dosed mice did not differ greatly from those in controls (i.e., 3/20, 9/50, and 6/48 in control, low dose, and high dose males, respectively; and 0/19, 1/49, and 1/48 in control, low dose, and high dose females). Among both sexes of mice, there was a significant positive association between the concentrations of the chemical administered and the incidences of a combination of hepatocellular adenomas and hepatocellular carcinomas. For

male mice, the Fisher exact comparisons were not significant; however, for females, both the high dose to control and the low dose to control comparisons were significant.

In both sexes of both species nonneoplastic proliferative lesions of the thyroid occurred in dosed animals but not in any of the controls.

Under the conditions of this bioassay, 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic in Fischer 344 rats, inducing thyroid follicular-cell carcinomas in both males and females. Administration of the compound was carcinogenic in female B6C3F1 mice, inducing liver neoplasms. There was no conclusive evidence that 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic in male B6C3F1 mice.

TABLE OF CONTENTS

	<u>Page</u>
I. INTRODUCTION	1
II. MATERIALS AND METHODS	5
A. Chemicals	5
B. Dietary Preparation	5
C. Animals	6
D. Animal Maintenance	6
E. Selection of Initial Concentrations	8
F. Experimental Design	10
G. Clinical and Histopathologic Examinations	13
H. Data Recording and Statistical Analyses	14
III. CHRONIC TESTING RESULTS: RATS	21
A. Body Weights and Clinical Observations	21
B. Survival	21
C. Pathology	21
D. Statistical Analyses of Results	27
IV. CHRONIC TESTING RESULTS: MICE	39
A. Body Weights and Clinical Observations	39
B. Survival	39
C. Pathology	42
D. Statistical Analyses of Results	44
V. DISCUSSION	51
VI. BIBLIOGRAPHY	55
APPENDIX A	
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 4,4'-METHYLENEBIS(N,N- DIMETHYL)BENZENAMINE	A-1
APPENDIX B	
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 4,4'-METHYLENEBIS(N,N- DIMETHYL)BENZENAMINE	B-1
APPENDIX C	
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 4,4'-METHYL- ENE BIS(N,N-DIMETHYL)BENZENAMINE	C-1

TABLE OF CONTENTS (Concluded)

	<u>Page</u>
APPENDIX D SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 4,4'-METHYL- ENEBIS(N,N-DIMETHYL)BENZENAMINE	D-1

1

LIST OF ILLUSTRATIONS

<u>Figure Number</u>		<u>Page</u>
1	CHEMICAL STRUCTURE OF 4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE	2
2	GROWTH CURVES FOR 4,4'-METHYLENEBIS(N,N- DIMETHYL)BENZENAMINE CHRONIC STUDY RATS	22
3	SURVIVAL COMPARISONS OF 4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE CHRONIC STUDY RATS	23
4	GROWTH CURVES FOR 4,4'-METHYLENEBIS(N,N- DIMETHYL)BENZENAMINE CHRONIC STUDY MICE	40
5	SURVIVAL COMPARISONS OF 4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE CHRONIC STUDY MICE	41

LIST OF TABLES

<u>Table Number</u>		<u>Page</u>
1	DESIGN SUMMARY FOR FISCHER 344 RATS-- 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE FEEDING EXPERIMENT	11
2	DESIGN SUMMARY FOR B6C3F1 MICE--4,4'-METHYL- ENEBS(N,N-DIMETHYL)BENZENAMINE FEEDING EXPERIMENT	12
3	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	28
4	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	32
5	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	45
6	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	47

LIST OF TABLES (Concluded)

<u>Table Number</u>		<u>Page</u>
A1	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	A-3
A2	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	A-7
B1	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	B-3
B2	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	B-6
C1	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	C-3
C2	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	C-6
D1	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	D-3
D2	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE	D-6

I. INTRODUCTION

4,4'-Methylenebis(N,N-dimethyl)benzenamine (Figure 1) (NCI No. C01990), a bicyclic aromatic amine and an intermediate in dye manufacture, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer observed among dye manufacturing industry workers (Anthony and Thomas, 1970; Wynder et al., 1963). The carcinogenicity of the structurally similar compounds 4,4'-methylenebis(2-chloroaniline) and 4,4'-methylenebis(2-methylaniline) (International Agency for Research on Cancer, 1974) was an additional factor in the selection of 4,4'-methylenebis(N,N-dimethyl)benzenamine for testing.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 4,4'-methylenebis(N,N-dimethyl)benzenamine.* It is also called 4,4'-methylenebis(N,N-dimethyl)aniline; tetramethyldiaminodiphenylmethane; 4,4'-bis(dimethylamino)diphenylmethane; tetra base; methane base; Michler's base; Michler's hydride; and Michler's methane.

4,4'-Methylenebis(N,N-dimethyl)benzenamine is an intermediate in the synthesis of at least 5 dyes: C.I. (Colour Index) Basic Yellow 2, C.I. Solvent Yellow 34, Methylene Red, C.I. Basic Orange 14, and C.I. Solvent Orange 15 (Society of Dyers and Colourists, 1956).

*The CAS registry number is 101-61-1.

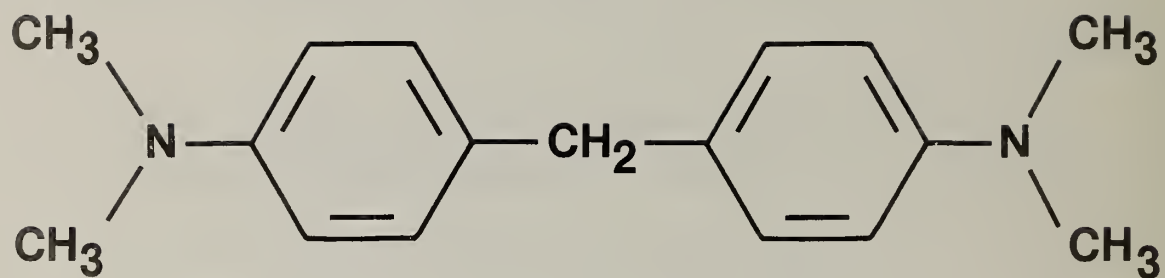


FIGURE 1
CHEMICAL STRUCTURE OF 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

4,4'-Methylenebis(N,N-dimethyl)benzenamine hydrochloride is used as an analytical reagent for the determination of lead (Windholz, 1976).

Specific production data for 4,4'-methylenebis(N,N-dimethyl)benzenamine are not available; however, this compound is produced in commercial quantities (in excess of 1000 pounds or \$1000 in value annually) by four U.S. companies (Stanford Research Institute, 1977). C.I. Basic Yellow 2 and C.I. Solvent Yellow 34, for which 4,4'-methylenebis(N,N-dimethyl)benzenamine is utilized as an intermediate, are also produced in commercial quantities in the United States (U.S. International Trade Commission, 1977).

The potential for exposure to 4,4'-methylenebis(N,N-dimethyl)benzenamine is greatest for workers in the dye and chemical manufacturing industries.

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade 4,4'-methylenebis(N,N-dimethyl)benzenamine was purchased from E.I. duPont de Nemours & Company, Wilmington, Delaware. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland. The experimentally determined melting point range, 85° to 87°C, was compared to the manufacturer's specification of 88°C. The results of infrared and nuclear magnetic resonance analyses were consistent with those expected based on the structure of the compound. Thin-layer chromatography was performed utilizing two solvent systems. Each plate was visualized with visible and ultraviolet light, iodine vapor, and ferric chloride-potassium ferricyanide spray. In all cases only one spot was observed.

Throughout this report, the term 4,4'-methylenebis(N,N-dimethyl)benzenamine is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). 4,4'-Methylenebis(N,N-dimethyl)benzenamine was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and a proper amount was blended with an aliquot of the feed using a mortar and pestle. Once visual homogeneity was attained, the mixture was placed in a

6 kg capacity Patterson-Kelley standard model twin-shell stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats were supplied by A. R. Schmidt, Madison, Wisconsin. Mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice, approximately 4 weeks old when received, were examined and any obviously ill or runted animals were killed. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

Animals were housed by species in rooms maintained at 22° to 26°C and 45 to 55 percent relative humidity. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia)

at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

Animal species were housed in separate rooms. Rats were housed four per cage by sex and mice were housed five per cage by sex. Polycarbonate cages (Lab Products, Inc., Garfield, New Jersey), which were suspended from aluminum racks, were used for dosed and control animals. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding (Ab-sorb-dri® hardwood chip bedding, Wilner Wood Products Company, Norway, Maine) were provided twice weekly.

Acidulated water (pH 2.5) was supplied to animals in water bottles which were changed and washed twice weekly. Sipper tubes were washed at weekly intervals. During the period of chemical administration, dosed and control animals received treated or untreated Wayne Lab-Blox® meal as appropriate. The feed was supplied in hanging stainless steel hoppers which were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

Dosed and control rats were housed in a room with other rats receiving diets containing* p-quinone dioxime (105-11-3); and NTA

*CAS registry numbers are given in parentheses.

trisodium salt (5064-31-3); and other rats intubated with styrene (100-42-5).

Dosed and control mice were housed in a room with other mice receiving diets containing Michler's ketone (90-94-8); 2-nitro-p-phenylenediamine (5307-14-2); p-chloroaniline (106-47-8); 5-chloro-o-toluidine (95-79-4); N-phenyl-p-phenylenediamine hydrochloride (2198-59-6); l-phenyl-2-thiourea (103-85-5); trimethylthiourea (2489-77-2); dibutyltin diacetate (1067-33-0); and 3-chloro-p-toluidine (95-74-9).

E. Selection of Initial Concentrations

To establish the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Rats were distributed among six groups, each consisting of five males and five females. 4,4'-Methylenebis(N,N-dimethyl)benzenamine was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 145, 315, 680, 1466 and 3155 ppm. The remaining rat group served as a control group, receiving only the basal laboratory diet.

Mice were distributed among eleven groups, each consisting of five males and five females. 4,4'-Methylenebis(N,N-dimethyl)benzenamine was incorporated into the basal laboratory diet and supplied ad libitum to nine of the eleven mouse groups in concentrations of 370, 550, 810, 1180, 1740, 3750, 5500, 8080 and 11,830 ppm. The two

remaining mouse groups served as control groups, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 4 weeks, followed by a 2-week observation period during which all animals were fed the basal laboratory diet. Individual body weights were recorded twice weekly throughout the study. Upon termination of the study all survivors were euthanized and necropsied.

The following table indicates the mean body weight gain, relative to controls, survival, and incidence of rough hair and arched backs observed in each of the rat groups at the end of the subchronic test.

RAT SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)*		Survival**		Observation of Rough Hair and Arched Backs**	
	Males	Females	Males	Females	Males	Females
3155	-135	-61	5/5	5/5	5/5	0/5
1466	-107	-49	5/5	5/5	0/5	0/5
680	- 37	-11	5/5	5/5	0/5	0/5
315	- 13	- 1	5/5	5/5	0/5	0/5
145	- 26	+ 6	5/5	5/5	0/5	0/5
0	--	--	5/5	5/5	0/5	0/5

The high concentration selected for administration to dosed rats in the chronic bioassay was 750 ppm.

*+ is indicative of mean body weight gain greater than that of controls.

- is indicative of mean body weight gain less than that of controls.

**Number of animals observed/number of animals originally in group.

The following table indicates the mean body weight gain, relative to controls, and survival observed in each of the mouse groups at the end of the subchronic test.

MOUSE SUBCHRONIC STUDY RESULTS

<u>ppm</u>	<u>Mean Body Weight Gain (%)*</u>		<u>Survival</u>	
	<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>
11,830	- 2	- 1	5/5	5/5
8,080	+ 3	- 2	5/5	5/5
5,500	-12	- 5	5/5	5/5
3,750	- 4	- 4	5/5	5/5
1,740	- 2	-10	5/5	5/5
1,180	- 4	- 8	5/5	5/5
810	- 1	+ 2	5/5	5/5
550	+ 6	- 8	5/5	5/5
370	+ 3	- 4	5/5	5/5
0	--	--	10/10	10/10

No other clinical abnormalities which could be attributed to administration of the compound were observed. The high concentration selected for administration to dosed mice in the chronic bioassay was 2500 ppm.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

*+ is indicative of mean body weight gain greater than that of controls.

- is indicative of mean body weight gain less than that of controls.

**Number of animals observed/number of animals originally in group.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4,4'-METHYLENEBIS (N,N-DIMETHYL) BENZENAMINE CONCENTRATION ^a	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
<u>MALE</u>				
CONTROL	20	0		104
LOW DOSE	50	375 0	59	45
HIGH DOSE	50	750 0	59	45
<u>FEMALE</u>				
CONTROL	20	0		104
LOW DOSE	50	375 0	59	45
HIGH DOSE	50	750 0	59	45

^aConcentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE CONCENTRATION ^a	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
<u>MALE</u>				
CONTROL	20	0		91
LOW DOSE	50	1250 0	78	13
HIGH DOSE	50	2500 0	78	13
<u>FEMALE</u>				
CONTROL	20	0		91
LOW DOSE	50	1250 0	78	13
HIGH DOSE	50	2500 0	78	13

^aConcentrations given in parts per million.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed rats were supplied with diets containing 750 and 375 ppm 4,4'-methylenebis(N,N-dimethyl)benzenamine for 59 weeks followed by a 45-week observation period, when no test chemicals were used. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed mice were supplied with diets containing 2500 and 1250 ppm 4,4'-methylenebis-(N,N-dimethyl)benzenamine for 78 weeks followed by a 13-week observation period, when no test chemicals were used. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights were recorded once a week for the first 6 weeks, every 2 weeks for the next 12 weeks, and at monthly intervals thereafter. All animals were inspected twice daily.

All moribund animals, animals that developed large, palpable masses that jeopardized their health, or animals that survived until the end of the bioassay were euthanized using carbon dioxide.

Necropsies were immediately performed on these animals and on all animals found dead during the bioassay. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were recorded in each group at the time that the test was initiated.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System

(Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which

that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k , are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P -value for any comparison be less than or equal to $0.05/k$. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P -values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test

determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed

and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower

limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

High dose male rats evidenced slight mean body weight depression in comparison with their controls after week 30. Slight dose-related mean body weight depression was apparent in female rats after week 35 (Figure 2).

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 4,4'-methylenebis(N,N-dimethyl)benzenamine-dosed groups are shown in Figure 3. The Tarone test for association between dosage and mortality was not significant for either males or females.

There were adequate numbers of male rats at risk from late-developing tumors, as 88 percent (44/50) of the high dose, 78 percent (39/50) of the low dose, and 80 percent (16/20) of the controls survived on test for at least 104 weeks.

There were adequate numbers of female rats at risk from late-developing tumors, as 74 percent (37/50) of the high dose, 82 percent (41/50) of the low dose, and 85 percent (17/20) of the controls survived on test for at least 104 weeks.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).

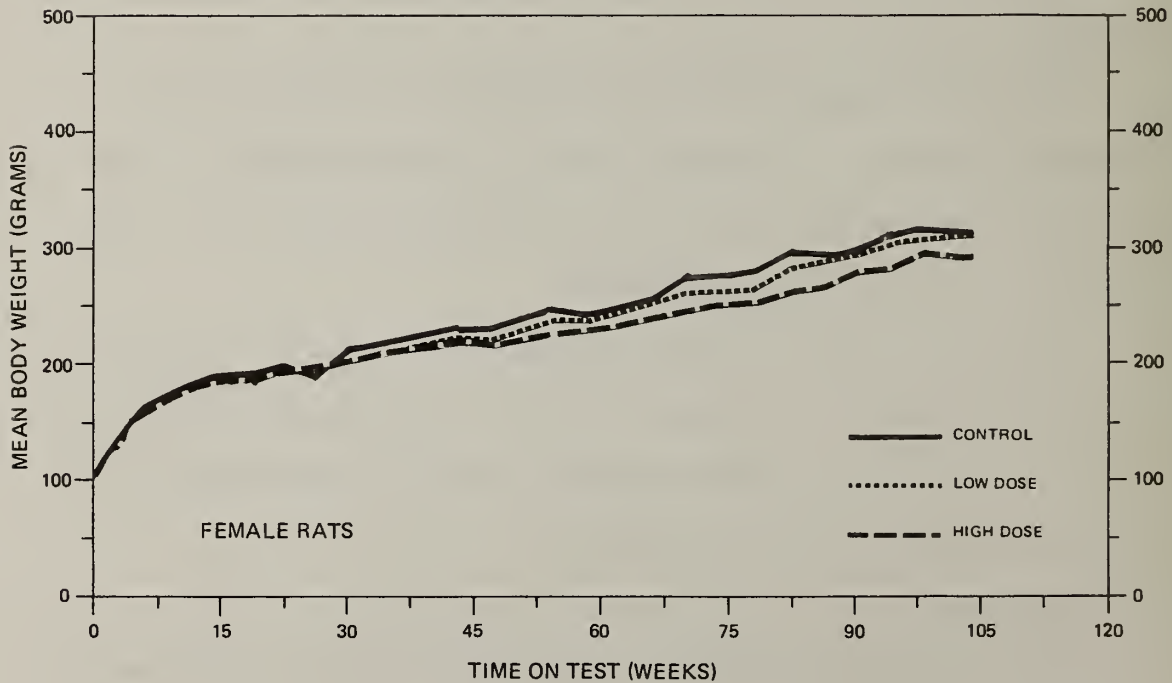
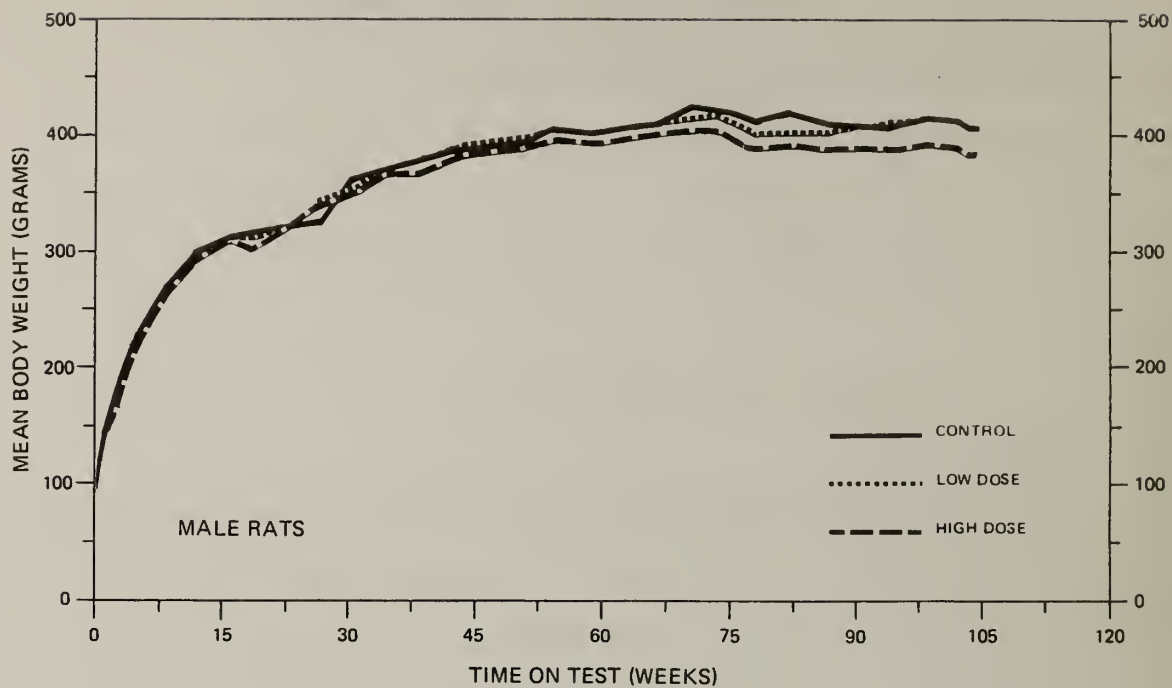


FIGURE 2
GROWTH CURVES FOR 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
CHRONIC STUDY RATS

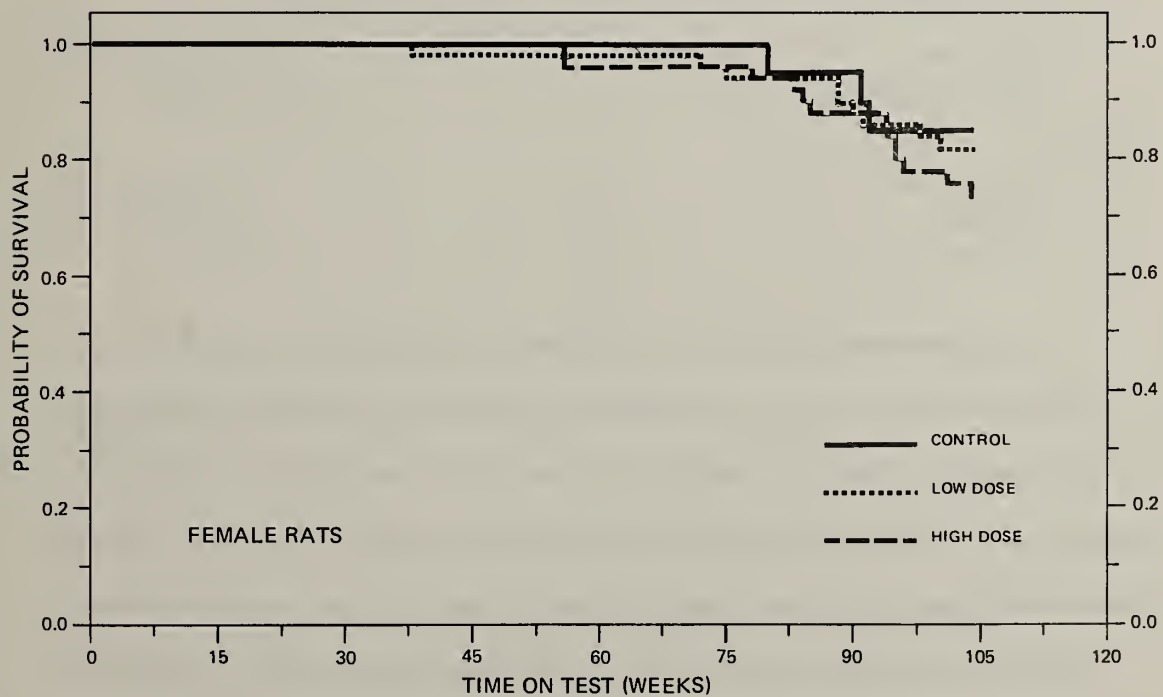
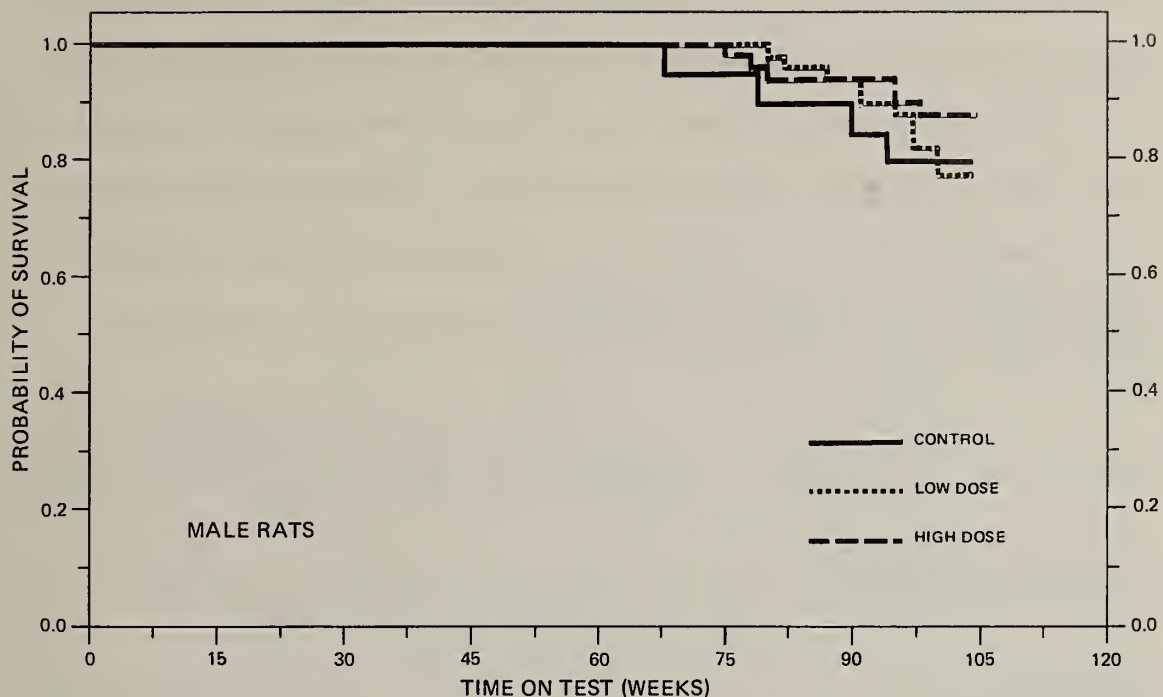


FIGURE 3
SURVIVAL COMPARISONS OF 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
CHRONIC STUDY RATS

The occurrence of follicular neoplasms of the thyroid gland, both of male and female rats, was related to the administration of 4,4'-methylenebis(N,N-dimethyl)benzenamine. The number of animals with follicular cysts, hyperplasia, adenoma, carcinoma, and C-cell hyperplasia was also increased. The incidences of these neoplastic and nonneoplastic lesions are summarized in the following table:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. of Animals with Thyroids Examined Histopathologically	(18)	(50)	(46)	(20)	(46)	(45)
<u>Follicular:</u>						
Cyst	0	4	3	0	3	1
Hyperplasia	0	0	7	0	1	5
Adenoma	0	0	13	0	1	13
Carcinoma	1	4	21	0	3	23
Neoplasm NOS	0	0	1	0	0	1
<u>C-Cell:</u>						
Hyperplasia	0	5	1	0	1	1
Adenoma	2	0	3	2	1	1
Carcinoma	0	2	1	0	1	0

A markedly distended follicle with eosinophilic or pale colloid and normal epithelium was considered a follicular cyst. Follicular hyperplasia was either localized or diffuse. Irregular papillary ingrowths of the epithelium resulted in follicles of varying sizes. The follicular cells were large, and the nuclei were hyperchromatic.

Follicular neoplasms of the thyroid gland were generally well-vascularized. Many arteries, particularly those in the capsule, were

sclerotic and occluded. The fibrous capsule was prominent in animals with this neoplasm. Some of the morphologic features of these neoplasms (adenoma and carcinoma) are recorded in the following table:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. of Animals with Thyroids Examined Histopathologically	(18)	(50)	(46)	(20)	(46)	(45)
<u>Follicular:</u>						
Adenoma	0	0	13	0	1	13
Carcinoma	1	4	21	0	3	23
<u>Lobes Involved:</u>						
One	1	4	21	0	4	9
Both	0	1	13	0	0	27
<u>Infiltration:</u>						
Capsule	1	4	21	0	3	22
Blood Vessel	0	0	2	0	0	6
<u>Compression:</u>						
Trachea	0	0	6	0	0	5
Esophagus	0	0	1	0	0	2

Follicular adenoma involved a part or an entire lobe of the gland and compressed the adjacent areas of tissue. Although circumscribed, not all adenomas were encapsulated. The morphologic features of the adenomas were different from either normal or hyperplastic tissue, in that they were cellular with piling of cells in some areas. Both macro- and micro-follicular variants were recognized. The follicular pattern was common in many adenomas, and the

papillary pattern in others. The epithelial cells were cuboidal or columnar. Cytoplasm was basophilic, and the nuclei were hyperchromatic. Mitotic figures were infrequent. Infiltration of the tumor into the capsule or blood vessels, together with distortion of architecture and back-to-back cellular arrangement, were considered the cardinal features of a follicular carcinoma. The carcinoma either involved one or both lobes and compressed the trachea or esophagus in a few animals.

Both follicular and follicular-papillary arrangements of cells were common in many tumors. The colloid was eosinophilic in large follicles and unstained in others. A pleomorphism in follicular size was evident. Back-to-back arrangement of cells with scant stroma was found in many areas. The cells were either columnar or cuboidal with a basophilic cytoplasm and hyperchromatic nuclei. Mitotic figures were not numerous.

Areas of necrosis and cystic degeneration were seen in many tumors. Foci of mineralization, clumps of golden-brown pigment, and cholesterol clefts were additional features in areas of degeneration. In a few tumors, the stroma was hyalinized and there was a stromal reaction (as evidenced by proliferating fibroblasts). Squamous metaplasia was noticed in one tumor. Follicular carcinoma in one high dose rat had metastasized to the lung.

Hyperplasia and neoplasia of the C-cells of the thyroid gland occurred both in control and dosed rats, and probably was not compound-related.

The nonneoplastic lesions in the dosed rats did not appear to be related to the feeding of the chemical.

Based on the results of this pathology examination, 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic to male and female Fischer 344 rats, inducing follicular-cell neoplasms of the thyroid gland under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 4,4'-methylenebis(N,N-dimethyl)benzenamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male rats, the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of follicular-cell carcinomas of the thyroid. This was supported by a significant ($P = 0.002$) positive high dose to control Fisher exact comparison. The test for departure from linear trend was also significant at this site. The Cochran-Armitage test also indicated a significant ($P < 0.001$) positive association between dose and the combined incidence of follicular-cell carcinomas or follicular-cell

TABLE 3
ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE^a

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma ^b	2/20(0.10)	1/50(0.02)	0/50(0.00)
P Values ^c	P = 0.035(N)	N.S.	N.S.
Relative Risk (Control) ^d	---	0.200	0.000
Lower Limit	---	0.004	0.000
Upper Limit	---	3.681	1.345
Weeks to First Observed Tumor	68	97	---
Liver: Hepatocellular Carcinoma ^b	2/19(0.11)	0/50(0.00)	0/49(0.00)
P Values ^c	P = 0.023(N)	N.S.	N.S.
Departure from Linear Trend ^e	P = 0.036	---	---
Relative Risk (Control) ^d	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.278	1.303
Weeks to First Observed Tumor	104	---	---
Liver: Hepatocellular Carcinoma or Neoplastic Nodule ^b	3/19(0.16)	5/50(0.10)	3/49(0.06)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.633	0.388
Lower Limit	---	0.141	0.058
Upper Limit	---	3.807	2.710
Weeks to First Observed Tumor	104	104	98

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma ^b	2/19(0.11)	12/47(0.26)	14/48(0.29)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	2.426	2.771
Lower Limit	---	0.625	0.739
Upper Limit	---	21.038	23.650
Weeks to First Observed Tumor	104	97	80
Adrenal: Pheochromocytoma ^b	1/20(0.05)	3/47(0.06)	5/48(0.10)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	1.277	2.083
Lower Limit	---	0.112	0.259
Upper Limit	---	65.563	96.358
Weeks to First Observed Tumor	104	82	104
Thyroid: Follicular-Cell Carcinoma ^b	1/18(0.06)	4/50(0.08)	21/46(0.46)
P Values ^c	P < 0.001	N.S.	P = 0.002
Departure from Linear Trend ^e	P = 0.031	---	---
Relative Risk (Control) ^d	---	1.440	8.217
Lower Limit	---	0.159	1.532
Upper Limit	---	69.469	326.674
Weeks to First Observed Tumor	104	95	95

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma ^b	1/18(0.06)	4/50(0.08)	34/46(0.74)
P Values ^c	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend ^e	P < 0.001	---	---
Relative Risk (Control) ^d	---	1.440	13.304
Lower Limit	---	0.159	2.653
Upper Limit	---	69.469	496.812
Weeks to First Observed Tumor	104	95	80
Thyroid: C-Cell Carcinoma or C-Cell Adenoma ^b	2/18(0.11)	2/50(0.04)	4/46(0.09)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.360	0.783
Lower Limit	---	0.029	0.127
Upper Limit	---	4.740	8.213
Weeks to First Observed Tumor	90	104	104
Testis: Interstitial-Cell Tumor ^b	20/20(1.00)	47/49(0.96)	45/49(0.92)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.959	0.918
Lower Limit	---	0.000	0.000
Upper Limit	---	1.111	1.250
Weeks to First Observed Tumor	68	80	78

TABLE 3 (CONCLUDED)

- ^aTreated groups received doses of 375 or 750 ppm in feed.
- ^bNumber of tumor-bearing animals/number of animals examined at site (proportion).
- ^cThe probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.
- ^dThe 95% confidence interval on the relative risk of the treated group to the control group.
- ^eThe probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL) BENZENAMINE^a

TOPOGRAPHY: MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma ^b	1/20(0.05)	3/49(0.06)	1/50(0.02)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	1.224	0.400
Lower Limit	---	0.108	0.005
Upper Limit	---	62.958	30.802
Weeks to First Observed Tumor	104	104	104
Liver: Neoplastic Nodule ^b	0/20(0.00)	0/49(0.00)	4/47(0.09)
P Values ^c	P = 0.036	N.S.	N.S.
Relative Risk (Control) ^d	---	---	Infinite
Lower Limit	---	---	0.411
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	104
Pituitary: Chromophobe Adenoma ^b	11/20(0.55)	18/47(0.38)	21/48(0.44)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.696	0.795
Lower Limit	---	0.410	0.482
Upper Limit	---	1.354	1.507
Weeks to First Observed Tumor	80	91	84

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY		CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Carcinoma ^b		0/20(0.00)	3/46(0.07)	23/45(0.51)
P Values ^c		P < 0.001	N.S.	P < 0.001
Departure from Linear Trend ^e		P = 0.021	---	---
Relative Risk (Control) ^d		---	Infinitive	Infinitive
Lower Limit		---	0.272	3.492
Upper Limit		---	Infinitive	Infinitive
Weeks to First Observed Tumor		---	104	83
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma ^b		0/20(0.00)	4/46(0.09)	36/45(0.80)
P Values ^c		P < 0.001	N.S.	P < 0.001
Departure from Linear Trend ^e		P = 0.001	---	---
Relative Risk (Control) ^d		---	Infinitive	Infinitive
Lower Limit		---	0.420	5.720
Upper Limit		---	Infinitive	Infinitive
Weeks to First Observed Tumor		---	104	83
Thyroid: C-Cell Carcinoma or C-Cell Adenoma ^b		2/20(0.10)	2/46(0.04)	1/45(0.02)
P Values ^c		N.S.	N.S.	N.S.
Relative Risk (Control) ^d		---	0.435	0.222
Lower Limit		---	0.034	0.004
Upper Limit		---	5.721	4.077
Weeks to First Observed Tumor		104	104	104

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma ^b	1/20(0.05)	5/50(0.10)	8/50(0.16)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	2.000	3.200
Lower Limit	---	0.249	0.482
Upper Limit	---	92.596	138.771
Weeks to First Observed Tumor	104	104	101
Mammary Gland: Fibroadenoma, Adenoma NOS or Adenocarcinoma NOS ^b	1/20(0.05)	7/50(0.14)	9/50(0.18)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	2.800	3.600
Lower Limit	---	0.403	0.561
Upper Limit	---	123.407	154.106
Weeks to First Observed Tumor	104	90	85
Uterus: Endometrial Stromal Polyp ^b	1/20(0.05)	3/49(0.06)	3/49(0.06)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	1.224	1.224
Lower Limit	---	0.108	0.108
Upper Limit	---	62.958	62.958
Weeks to First Observed Tumor	104	104	104

TABLE 4 (CONCLUDED)

- ^aTreated groups received doses of 375 or 750 ppm in feed.
- ^bNumber of tumor-bearing animals/number of animals examined at site (proportion).
- ^cThe probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.
- ^dThe 95% confidence interval on the relative risk of the treated group to the control group.
- ^eThe probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

adenomas of the thyroid. The Fisher exact high dose to control comparison was significant ($P < 0.001$), as was the test for departure from linear trend.

In female rats, the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of follicular-cell carcinomas of the thyroid. This was supported by a significant ($P < 0.001$) positive Fisher exact test comparing the high dose group to the control group. The test for departure from linear trend was also significant. As in males, the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the combined incidence of follicular-cell carcinomas or follicular-cell adenomas of the thyroid. This was supported by a significant ($P < 0.001$) positive high dose to control Fisher exact comparison. The test for departure from linear trend was also significant at this site. Also in female rats, the Cochran-Armitage test indicated a significant ($P = 0.036$) positive association between dose and the incidence of neoplastic nodules of the liver. This result, however, was not supported by either of the Fisher exact tests.

Based on these statistical results, the administration of 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic to both male and female Fischer 344 rats under the conditions of this bioassay, causing both follicular-cell carcinomas of the thyroid and a combination of follicular-cell carcinomas or follicular-cell adenomas of the thyroid in rats of both sexes.

In male rats, the Cochran-Armitage test indicated a significant negative association both between dose and the incidence of fibromas of the subcutaneous tissue and between dose and the incidence of hepatocellular carcinomas. In the latter case, the test for departure from linear trend was also significant.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

The mean body weights of dosed male mice were considerably lower than that of the controls after week 30. Dose-related mean body weight depression was apparent in female mice after week 30 (Figure 4).

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 4,4'-methylenebis(N,N-dimethyl)benzenamine-dosed groups are shown in Figure 5. The Tarone test for association between dosage and mortality was not significant for either male or female mice, but the test for departure from linear trend was significant for males.

There were adequate numbers of male mice at risk from late-developing tumors, as 100 percent (50/50) of the high dose, 94 percent (47/50) of the low dose and 100 percent (20/20) of the controls survived on test until the termination of the study.

There were adequate numbers of female mice at risk from late-developing tumors, as 84 percent (42/50) of the high dose, 92 percent (46/50) of the low dose and 90 percent (18/20) of the controls survived on test until the termination of the study. Two high dose females and one low dose female were missing in week 14.

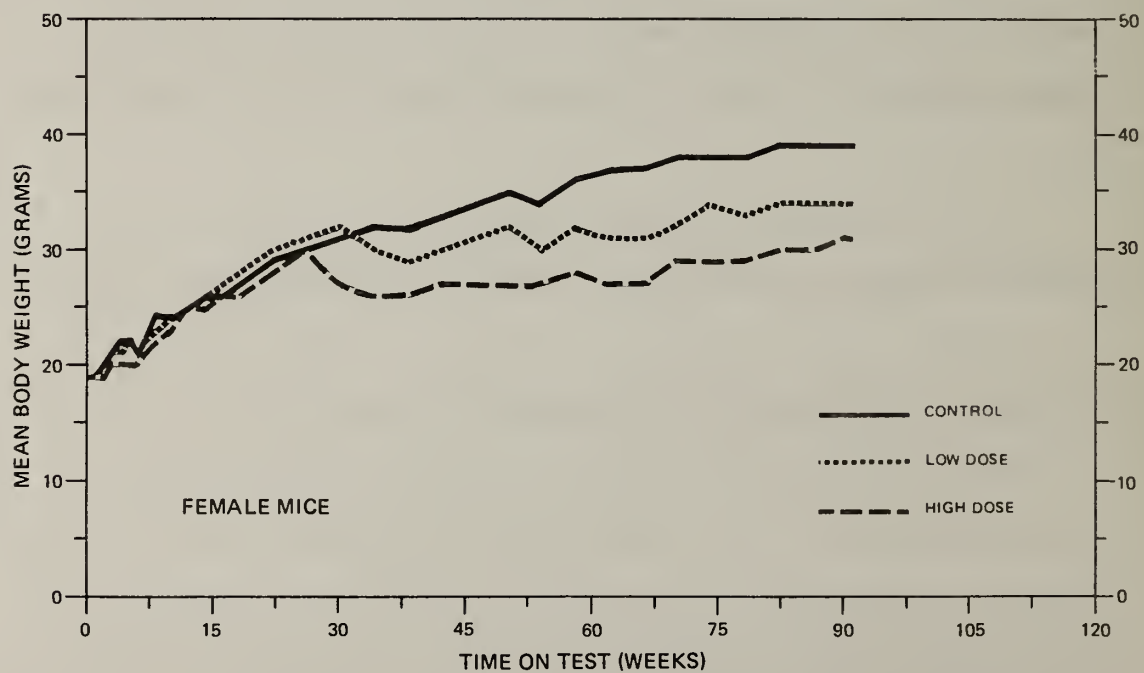
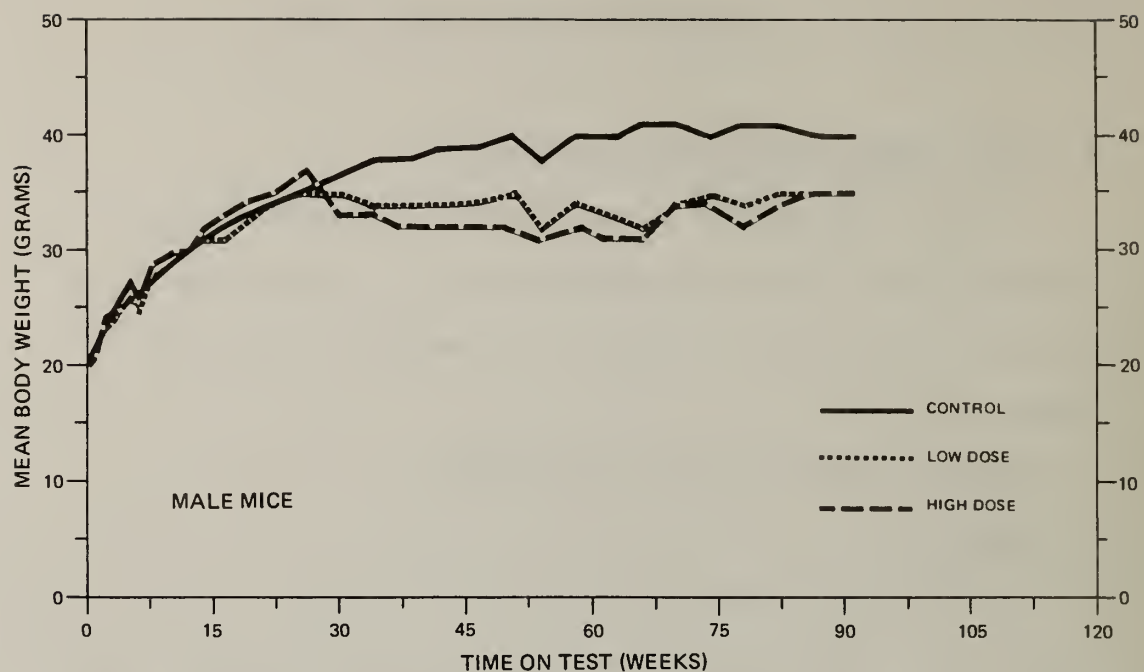


FIGURE 4
GROWTH CURVES FOR 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
CHRONIC STUDY MICE

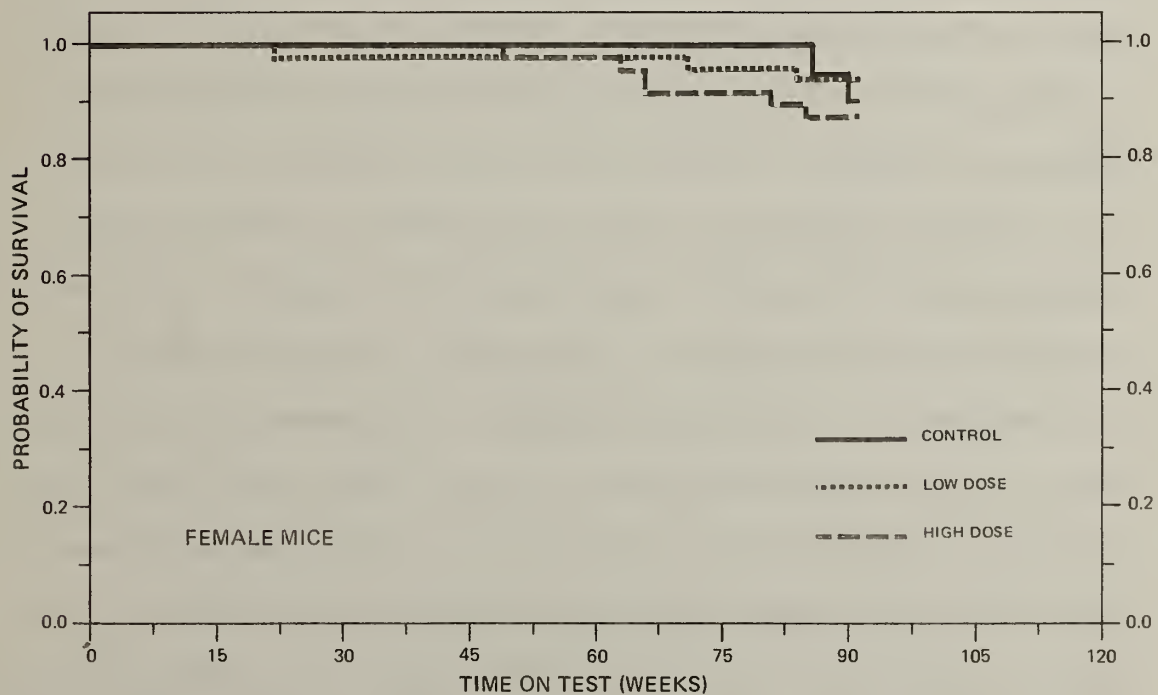
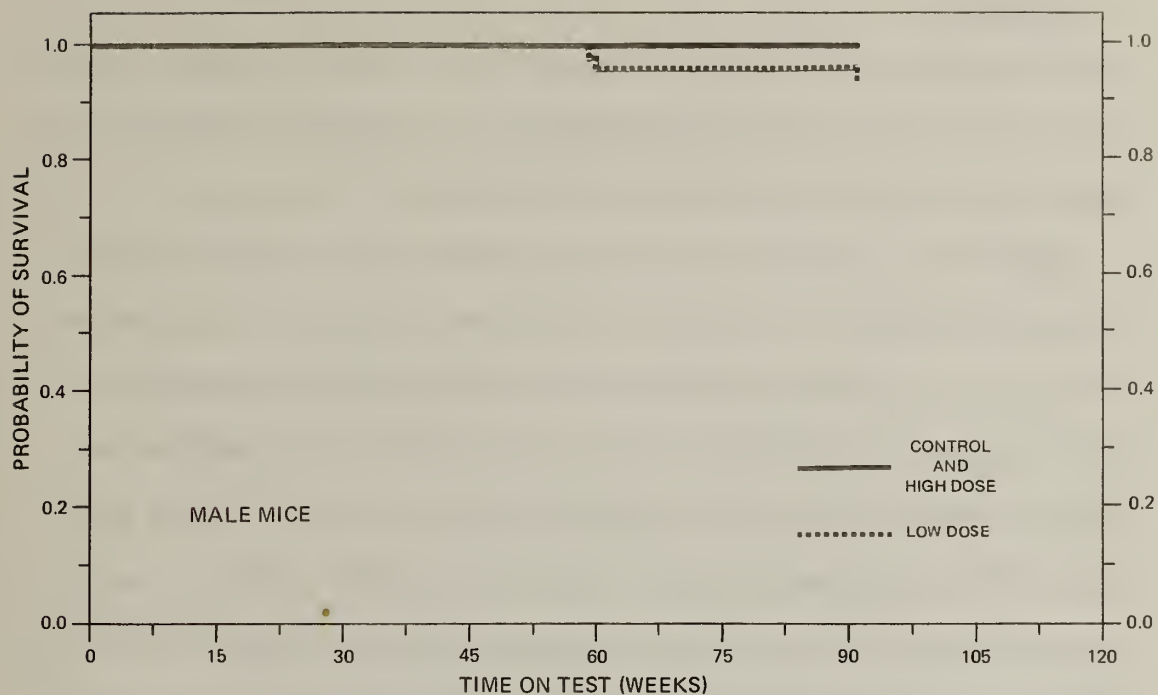


FIGURE 5
SURVIVAL COMPARISONS OF 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
CHRONIC STUDY MICE

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).

A variety of neoplasms was seen in both control and dosed mice. The majority appeared unrelated to chemical administration. However, there was an increased incidence of hepatocellular adenomas (i.e., 2/20 [10 percent], 3/50 [6 percent], and 16/48 [33 percent] in control, low dose, and high dose males, respectively, and 1/19 [5 percent], 18/49 [37 percent], and 22/48 [46 percent] in control, low dose, and high dose females) or hepatocellular carcinomas (i.e., 3/20 [15 percent], 9/50 [18 percent], and 6/48 [13 percent] in control, low dose, and high dose males, respectively, and 0/19, 1/49 [2 percent], and 1/48 [2 percent] in control, low dose, and high dose females) in dosed male and female mice. As indicated, most of the liver neoplasms observed in females were adenomas. Hepatocellular adenomas were usually small, roughly spherical expanding nodules of well-differentiated basophilic, eosinophilic, and vacuolated cells which compressed the adjacent parenchyma. Eosinophilic and vacuolated cells were larger than normal. Mitotic figures were rare. Some tumors contained a few cells with hyaline cytoplasmic droplets. Hepatocellular carcinomas were mostly solid tumors with areas of trabecular formation. Variable morphologic areas were common within the same tumor. Cytoplasmic vacuolation and hyaline inclusions,

along with small hyperbasophilic and large eosinophilic cells, occurred. Mitotic figures were often plentiful. Hepatocytes arranged in papillary formations and extensive tumor necrosis were also seen. Foci of cytoplasmic change occurred in an elevated incidence in the livers of 6/49 (12 percent) low dose and 7/48 (15 percent) high dose female mice, a finding which is consistent with the increased incidence of hepatic tumors induced by the test compound.

Nonneoplastic proliferative lesions induced in mice were found in the thyroid. Thyroid papillary hyperplasia occurred in 10/45 (22 percent) low dose male, 13/49 (27 percent) high dose male, 7/43 (16 percent) low dose female, and 6/43 (14 percent) high dose female mice. Adenomatous goiter occurred in 4/45 (9 percent) low dose male, 22/49 (45 percent) high dose male, 25/43 (58 percent) low dose female, and 35/43 (81 percent) high dose female mice. Two high dose males were diagnosed as having colloid goiter, a stage of adenomatous goiter, which would make the total goiter incidence 24/49 (49 percent) for this group. None of the types of lesions discussed above were observed in male or female controls. Papillary thyroid hyperplasia was characterized by enlarged follicles with hyperplastic papillae projecting into their lumen. This change was usually focal, involving a few follicles, or had a patchy distribution throughout the thyroid. In some cases, the papillary hyperplasias resembled papillary adenomas. However, there was no connective tissue capsule and lesions were usually multifocal, labular or diffuse.

In adenomatous goiter there was diffuse distention of follicles with colloid, often with considerable variation in follicular size. The goiters in female mice were more severe than those in males. Some thyroids had other lesions such as hemosiderin deposition, sloughed follicular cells, calcified debris in the colloid, and stromal fibrosis.

Other inflammatory and degenerative lesions were seen which commonly occur in aging mice of this strain. These occurred in approximately equal numbers compared to controls and thus were not considered compound-related.

Based on the results of this pathology examination, 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic to B6C3F1 mice, inducing hepatocellular neoplasms under the conditions of this bioassay. The compound also induced hyperplasias of thyroid follicular cells in male and female B6C3F1 mice.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 4,4'-methylenebis(N,N-dimethyl)benzenamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male mice, the Cochran-Armitage test indicated a significant ($P = 0.025$) positive association between dose and the combined

TABLE 5
ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL) BENZENAMINE^a

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma ^b	1/20(0.05)	6/49(0.12)	7/50(0.14)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	2.449	2.800
Lower Limit	---	0.332	0.403
Upper Limit	---	110.166	123.407
Weeks to First Observed Tumor	91	91	91
All Sites: Hemangioma or Hemangiosarcoma ^b	0/20(0.00)	5/50(0.10)	5/50(0.10)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.525	0.525
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	91
Liver: Hepatocellular Carcinoma ^b	3/20(0.15)	9/50(0.18)	6/48(0.13)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	1.200	0.833
Lower Limit	---	0.346	0.204
Upper Limit	---	6.408	4.799
Weeks to First Observed Tumor	91	91	91

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma ^b	5/20(0.25)	12/50(0.24)	22/48(0.46)
P Values ^c	P = 0.025	N.S.	N.S.
Relative Risk (Control) ^d	---	0.960	1.833
Lower Limit	---	0.376	0.822
Upper Limit	---	3.124	5.424
Weeks to First Observed Tumor	91	91	91

^aTreated groups received doses of 1250 or 2500 ppm in feed.

^bNumber of tumor-bearing animals/number of animals examined at site (proportion).

^cThe probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

^dThe 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 6
ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE^a

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma ^b	0/20(0.00)	3/48(0.06)	0/47(0.00)
P Values ^c	N.S.	N.S.	N.S.
Departure from Linear Trend ^e	P = 0.046	---	---
Relative Risk (Control) ^d	---	Infinite	---
Lower Limit	---	0.261	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	91	---
Hematopoietic System: Leukemia or Malignant Lymphoma ^b	5/20(0.25)	6/49(0.12)	7/48(0.15)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.490	0.583
Lower Limit	---	0.146	0.187
Upper Limit	---	1.842	2.109
Weeks to First Observed Tumor	86	84	81
All Sites: Hemangioma or Hemangiosarcoma ^b	0/20(0.00)	1/49(0.02)	3/48(0.06)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.023	0.261
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	85

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma ^b	1/19(0.05)	19/49(0.39)	23/48(0.48)
P Values ^c	P = 0.002	P = 0.005	P = 0.001
Relative Risk (Control) ^d	---	7.367	9.104
Lower Limit	---	1.343	1.704
Upper Limit	---	296.332	360.488
Weeks to First Observed Tumor	91	84	91

^aTreated groups received doses of 1250 or 2500 ppm in feed.

^bNumber of tumor-bearing animals/number of animals examined at site (proportion).

^cThe probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group than in the control group.

^dThe 95% confidence interval on the relative risk of the treated group to the control group.

^eThe probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

incidence of hepatocellular carcinomas or hepatocellular adenomas. However, neither of the Fisher exact comparisons were significant. No other statistical tests for tumor incidence were significant at any site in male mice.

In female mice, the Cochran-Armitage test indicated a significant ($P = 0.002$) positive association between dose and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas. This result was supported both by a significant ($P = 0.001$) positive high dose to control Fisher exact comparison and by a significant ($P = 0.005$) positive low dose to control comparison. No other statistical tests were significant at any site in female mice.

Based on these statistical results, the administration of 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic to female B6C3F1 mice, causing a combination of hepatocellular carcinomas or hepatocellular adenomas under the conditions of this bioassay.

V. DISCUSSION

There were no significant positive associations between the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered and mortality among rats or mice of either sex. The compound was administered for 59 weeks to rats and for 78 weeks to mice. Adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. There was slight dose-related mean body weight depression among female rats, the mean body weight of high dose male rats was slightly less than that for controls, and the mean body weights of dosed mice were significantly lower than their controls, indicating that the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered to these animals in this bioassay may have approximated the maximum tolerated concentrations.

For both male and female rats, there was a significant positive association between the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered and the incidences of follicular-cell carcinomas of the thyroid (i.e., 1/18, 4/50, and 21/46 in the control, low dose, and high dose males, respectively; and 0/20, 3/46, and 23/45 in the control, low dose, and high dose females, respectively). The high dose to control Fisher exact comparisons were also significant for each sex. These associations were apparent even though the compound was administered to the dosed rats for only 59 weeks instead of the usual 78 weeks.

Liver neoplasms were observed among male and female mice. There were elevated incidences of hepatocellular adenomas in dosed mice when compared to controls (i.e., 2/20, 3/50, and 16/48 in control, low dose, and high dose males, respectively; and 1/19, 18/49, and 22/48 in control, low dose, and high dose females). The incidences of hepatocellular carcinomas in dosed mice did not differ greatly from those in controls (i.e., 3/20, 9/50, and 6/48 in control, low dose, and high dose males, respectively; and 0/19, 1/49, and 1/48 in control, low dose, and high dose females). Among both sexes of mice, there was a significant positive association between the concentrations of the chemical administered and the incidences of a combination of hepatocellular adenomas and hepatocellular carcinomas. For male mice, the Fisher exact comparisons were not significant; however, for females, both the high dose to control and the low dose to control comparisons were significant.

In both sexes of both species, nonneoplastic proliferative lesions of the thyroid occurred in dosed animals but not in any of the controls. This may represent a toxic lesion in both species which is associated with cancer of the thyroid in the rat but not in the mouse.

Under the conditions of this bioassay, 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic in Fischer 344 rats, inducing thyroid follicular-cell carcinomas in both males and females. Administration of the compound was carcinogenic in female B6C3F1 mice,

inducing liver neoplasms. There was no conclusive evidence that 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic in male B6C3F1 mice.

VI. BIBLIOGRAPHY

- Anthony, H.M. and G.M. Thomas, "Tumors of the Urinary Bladder: An Analysis of the Occupations of 1,030 Patients in Leeds, England." Journal of the National Cancer Institute 45:879-895, 1970.
- Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.
- Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.
- Chemical Abstracts Service, The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.
- Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.
- Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.
- Gart, J.J., "The Comparison of Proportions: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.
- International Agency for Research on Cancer, IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man: Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds, and Miscellaneous Alkylating Agents, Volume 4. IARC, Lyon, France, 1974.
- Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.
- Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.
- Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.

- Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.
- Society of Dyers and Colourists, Colour Index, 2nd edition, Volume 3. Yorkshire, England, 1956.
- Stanford Research Institute, 1977 Directory of Chemical Producers, U.S.A. Menlo Park, California, 1977.
- Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.
- U.S. International Trade Commission, Synthetic Organic Chemicals: United States Production and Sales, 1976. USITC Publication 833, U.S. Government Printing Office, Washington, D.C., 1977.
- Windholtz, M., editor, The Merck Index: An Encyclopedia of Chemicals and Drugs, 9th edition. Merck and Company, Inc., Rahway, New Jersey, 1976.
- Wynder, E.L., J. Onderdonk, and N. Mantel, "An Epidemiological Investigation of Cancer of the Bladder." Cancer 16:1388-1407, 1963.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS
IN RATS TREATED WITH 4,4'-METHYLENEBIS
(N,N-DIMETHYL)BENZENAMINE

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
BASAL-CELL CARCINOMA	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SARCOMA, NOS	1 (5%)		
FIBROMA	2 (10%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMIA, NOS	1 (5%)	1 (2%)	
*HEMATOPOIETIC SYSTEM	(20)	(50)	(50)
NEOPLASM, NOS		2 (4%)	
#SPLEEN	(18)	(48)	(47)
HEMANGIOMA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(50)	(49)
NEOPLASTIC NODULE	1 (5%)	5 (10%)	3 (6%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
HEPATOCELLULAR CARCINOMA	2 (11%)		
*STOMACH SQUAMOUS CELL PAPILLOMA	(18) 1 (6%)	(49)	(47)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*PITUITARY CHROMOPHOBE ADENOMA	(19) 2 (11%)	(47) 12 (26%)	(48) 14 (29%)
*ADRENAL NEOPLASM, NOS PHEOCHROMOCYTOMA	(20) 1 (5%)	(47) 3 (6%)	(48) 1 (2%) 5 (10%)
*THYROID NEOPLASM, NOS FOLLICULAR-CELL ADENOMA FOLLICULAR-CELL CARCINOMA C-CELL ADENOMA C-CELL CARCINOMA	(18) 1 (6%) 2 (11%)	(50) 4 (8%) 2 (4%)	(46) 1 (2%) 13 (28%) 21 (46%) 3 (7%) 1 (2%)
*THYROID FOLLICLE NEOPLASM, NOS CYSTADENOMA, NOS	(18)	(50) 1 (2%)	(46) 1 (2%)
*PARATHYROID ADENOMA, NOS	(10)	(28) 1 (4%)	(10)
*PANCREATIC ISLETS ISLET-CELL ADENOMA	(16) 1 (6%)	(48) 1 (2%)	(43) 1 (2%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND ADENOMA, NOS	(20) 1 (5%)	(50) 1 (2%)	(50)
*TESTIS INTERSTITIAL-CELL TUMOR	(20) 20 (100%)	(49) 47 (96%)	(49) 45 (92%)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
NERVOUS SYSTEM			
*BRAIN ASTROCYTOMA	(20)	(50) 1 (2%)	(48)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*TUNICA VAGINALIS MESOTHELIOMA, NOS	(20)	(50) 1 (2%)	(50) 2 (4%)
ALL OTHER SYSTEMS			
SITE UNKNOWN NEOPLASM, NOS			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	10	4
MORIBUND SACRIFICE	1	1	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	39	44
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	50	50
TOTAL PRIMARY TUMORS	38	85	114
TOTAL ANIMALS WITH BENIGN TUMORS	20	50	48
TOTAL BENIGN TUMORS	31	68	83
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	9	22
TOTAL MALIGNANT TUMORS	6	9	22
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1	8	7
TOTAL UNCERTAIN TUMORS	1	8	9
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)
FIBROMA	1 (5%)		
LEIOMYOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	3 (6%)	1 (2%)
FOLLICULAR-CELL CARCINOMA, METAS			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA, NOS	1 (5%)	2 (4%)	1 (2%)
*MESENTERIC L. NODE	(15)	(43)	(45)
SARCOMA, NOS	1 (7%)		
CIRCULATORY SYSTEM *			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(20)	(49)	(47)
NEOPLASTIC NODULE			4 (9%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
LEIOMYOSARCOMA, INVASIVE		1 (2%)	
*STOMACH LEIOMYOSARCOMA	(19)	(49) 1 (2%)	(49)
*PEYERS PATCH SARCOMA, NOS	(19) 1 (5%)	(49)	(46)
*COLON SARCOMA, NOS	(18)	(43)	(42) 1 (2%)
URINARY SYSTEM			
*URINARY BLADDER TRANSITIONAL-CELL PAPILLOMA	(13)	(38)	(38) 1 (3%)
ENDOCRINE SYSTEM			
*PITUITARY CHROMOPHOBE ADENOMA	(20) 11 (55%)	(47) 18 (38%)	(48) 21 (44%)
*ADRENAL CORTICAL ADENOMA PHEOCHROMOCYTOMA	(20)	(49) 1 (2%)	(50) 1 (2%)
*THYROID NEOPLASM, NOS FOLLICULAR-CELL ADENOMA FOLLICULAR-CELL CARCINOMA C-CELL ADENOMA C-CELL CARCINOMA	(20) 2 (10%)	(46) 1 (2%) 3 (7%) 1 (2%) 1 (2%)	(45) 1 (2%) 13 (29%) 23 (51%) 1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND ADENOMA, NOS ADENOCARCINOMA, NOS FIBROADENOMA	(20) 1 (5%)	(50) 1 (2%) 1 (2%) 5 (10%)	(50) 2 (4%) 1 (2%) 8 (16%)
*CLITORAL GLAND ADENOMA, NOS	(20) 1 (5%)	(50) 2 (4%)	(50) 1 (2%)
*UTERUS ADENOMA, NOS	(20)	(49) 1 (2%)	(49)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
ENDOMETRIAL STROMAL POLYP	1 (5%)	3 (6%)	3 (6%)
NERVOUS SYSTEM			
*BRAIN ASTROCYTOMA	(20)	(49)	(50) 1 (2%)
SPECIAL SENSE ORGANS			
*EYELID SARCOMA, NOS	(20)	(50) 1 (2%)	(50)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA MESOTHELIOMA, NOS	(20)	(50) 1 (2%)	(50)
ALL OTHER SYSTEMS			
SITE UNKNOWN CARCINOMA, NOS	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	8	7
MORIBUND SACRIFICE		1	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	41	37
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	33	44
TOTAL PRIMARY TUMORS	22	48	85
TOTAL ANIMALS WITH BENIGN TUMORS	16	29	36
TOTAL BENIGN TUMORS	18	36	52
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	11	25
TOTAL MALIGNANT TUMORS	4	11	28
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		1	5
TOTAL UNCERTAIN TUMORS		1	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS
IN MICE TREATED WITH 4,4'-METHYLENEBIS
(N,N-DIMETHYL)BENZENAMINE

10
9
8
7
6
5
4
3
2
1

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	6 (12%)	7 (14%)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(15)	(43)	(44)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG. LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(48)
HEPATOCELLULAR ADENOMA	2 (10%)	3 (6%)	16 (33%)
HEPATOCELLULAR CARCINOMA	3 (15%)	9 (18%)	6 (13%)
SARCOMA, NOS			1 (2%)
HEMANGIOMA		2 (4%)	4 (8%)
HEMANGIOSARCOMA		2 (4%)	
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
*PANCREAS HEMANGIOSARCOMA	(20)	(49) 1 (2%)	(48)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*THYROID FOLLICULAR-CELL ADENOMA	(18) 1 (6%)	(45) 1 (2%)	(49)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS HEMANGIOSARCOMA	(20)	(50) 1 (2%)	(50)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
ADIPOSE TISSUE HEMANGIOMA			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH ^a		3	
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	20	47	50
ANIMAL MISSING			
^a INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	24	31
TOTAL PRIMARY TUMORS	7	27	37
TOTAL ANIMALS WITH BENIGN TUMORS	4	10	24
TOTAL BENIGN TUMORS	4	12	28
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	14	9
TOTAL MALIGNANT TUMORS	3	15	9
TOTAL ANIMALS WITH SECONDARY TUMORS [#]		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
[#] SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*LUNG	(20)	(48)	(47)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (6%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(48)
MALIGNANT LYMPHOMA, NOS	5 (25%)	3 (6%)	5 (10%)
LYMPHOCYTIC LEUKEMIA		1 (2%)	
*MEDIASTINUM	(20)	(49)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
*SPLEEN	(19)	(49)	(47)
HEMANGIOSARCOMA			1 (2%)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
*LIVER	(19)	(49)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
*ILEUM	(20)	(48)	(44)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
DIGESTIVE SYSTEM			
*LIVER	(19)	(49)	(48)
HEPATOCELLULAR ADENOMA	1 (5%)	18 (37%)	22 (46%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA			1 (2%)
*HEPATIC CAPSULE	(19)	(49)	(48)
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*ADRENAL	(18)	(44)	(38)
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(48)
ADENOCARCINOMA, NOS		1 (2%)	
*UTERUS	(19)	(49)	(47)
LEIOMYOSARCOMA			1 (2%)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(49)	(48)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH ^a	2	2	5
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	46	42
ANIMAL MISSING		1	2
^a INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	26	30
TOTAL PRIMARY TUMORS	6	31	37
TOTAL ANIMALS WITH BENIGN TUMORS	1	21	25
TOTAL BENIGN TUMORS	1	22	25
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	9	10
TOTAL MALIGNANT TUMORS	5	9	12
TOTAL ANIMALS WITH SECONDARY TUMORS [#]		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
[#] SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC
LESIONS IN RATS TREATED WITH 4,4'-METHYLENEBIS
(N,N-DIMETHYL)BENZENAMINE

TABLE CI
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE INFLAMMATION, NOS	(20)	(49)	(50) 3 (6%)
#LUNG INFLAMMATION, NOS	(20)	(49) 3 (6%)	(50)
PNEUMONIA, CHRONIC MURINE		1 (2%)	2 (4%)
REACTION, FOREIGN BODY			4 (8%)
HEMATOPOIETIC SYSTEM			
#SPLEEN INFARCT, NOS	(18) 1 (6%)	(48)	(47)
HEMATOPOIESIS		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM DEGENERATION, NOS	(19) 4 (21%)	(50) 8 (16%)	(50) 5 (10%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND HYPERPLASIA, FOCAL	(18)	(47) 1 (2%)	(49)
#LIVER INFLAMMATION, FOCAL	(19)	(50) 1 (2%)	(49)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (UNTE) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
NECROSIS, FOCAL		1 (2%)	2 (4%)
METAMORPHOSIS FATTY		2 (4%)	4 (8%)
BASOPHILIC CYTO CHANGE	2 (11%)	9 (18%)	11 (22%)
CLEAR-CELL CHANGE		1 (2%)	5 (10%)
*PANCREATIC ACINUS	(16)	(48)	(43)
ATROPHY, NOS	1 (6%)	2 (4%)	2 (5%)
*ILEUM	(18)	(48)	(44)
INFLAMMATION, NOS			1 (2%)
URINARY SYSTEM			
*KIDNEY	(19)	(49)	(49)
NEPHROPATHY	16 (84%)	45 (92%)	39 (80%)
DEGENERATION, CYSTIC		1 (2%)	
*URINARY BLADDER	(14)	(41)	(40)
INFLAMMATION, NOS			1 (3%)
ENDOCRINE SYSTEM			
*ADRENAL MEDULLA	(20)	(47)	(48)
HYPERPLASIA, NOS		2 (4%)	2 (4%)
HYPERPLASIA, FOCAL			2 (4%)
*THYROID	(18)	(50)	(46)
FOLLICULAR CYST, NOS		4 (8%)	3 (7%)
HYPERPLASIA, C-CELL		5 (10%)	1 (2%)
HYPERPLASIA, FOLLICULAR-CELL			7 (15%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
GALACTOCELE		1 (2%)	
*PREPUTIAL GLAND	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
NECROSIS, NOS	1 (5%)		
*TESTIS	(20)	(49)	(49)
ATROPHY, NOS	1 (5%)	3 (6%)	2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
HYPERPLASIA, INTERSTITIAL CELL			2 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
NONE			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
RESPIRATORY SYSTEM			
*LUNG	(20)	(49)	(50)
PNEUMONIA, CHRONIC MURINE			1 (2%)
REACTION, FOREIGN BODY	1 (5%)		3 (6%)
HEMATOPOIETIC SYSTEM			
*BONE MARROW	(17)	(40)	(40)
MYELOFIBROSIS		1 (3%)	
*SPLEEN	(20)	(49)	(47)
HEMATOPOIESIS	2 (10%)	6 (12%)	2 (4%)
CIRCULATORY SYSTEM			
*HEART	(20)	(49)	(50)
PERIVASCULITIS	1 (5%)		
*MYOCARDIUM	(20)	(49)	(50)
DEGENERATION, NOS	1 (5%)	3 (6%)	2 (4%)
DIGESTIVE SYSTEM			
*LIVER	(20)	(49)	(47)
INFLAMMATION, NOS	1 (5%)		
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
CHOLANGIOFIBROSIS			1 (2%)
NECROSIS, FOCAL	1 (5%)		1 (2%)
NECROSIS, CENTRAL		1 (2%)	
METAMORPHOSIS FATTY	2 (10%)	1 (2%)	2 (4%)
BASOPHILIC CYTO CHANGE	13 (65%)	38 (78%)	23 (49%)
CLEAR-CELL CHANGE		1 (2%)	
HEMATOPOIESIS		1 (2%)	
*STOMACH	(19)	(49)	(49)
HYPERPLASIA, BASAL CELL		1 (2%)	
ACANTHOSIS		1 (2%)	
URINARY SYSTEM			
*KIDNEY	(20)	(49)	(49)
NEPHROPATHY	5 (25%)	26 (53%)	19 (39%)
ENDOCRINE SYSTEM			
*ADRENAL MEDULLA	(20)	(49)	(50)
HYPERPLASIA, FOCAL	1 (5%)		
*THYROID	(20)	(46)	(45)
FOLLICULAR CYST, NOS		3 (7%)	1 (2%)
HYPERPLASIA, C-CELL		1 (2%)	1 (2%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	5 (11%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
GALACTOCELE	3 (15%)	5 (10%)	1 (2%)
*CLITORAL GLAND	(20)	(50)	(50)
NECROSIS, NOS		1 (2%)	
*UTERUS	(20)	(49)	(49)
HYDROMETRA		1 (2%)	
PYOMETRA		1 (2%)	
*UTERUS/ENDOMETRIUM	(20)	(49)	(49)
HYPERPLASIA, CYSTIC		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
NERVOUS SYSTEM			
#BRAIN HEMORRHAGE	(20)	(49) 1 (2%)	(50)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM NECROSIS, FAT		2	2
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED AUTO/NECROPSY/HISTO PERF		1	2 1
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC
LESIONS IN MICE TREATED WITH 4,4'-METHYLENEBIS
(N,N-DIMETHYL)BENZENAMINE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS	1 (5%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
GRANULOMA, FOREIGN BODY			1 (2%)
#LUNG/ALVEOLI	(20)	(49)	(50)
HEMORRHAGE		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(50)	(47)
ERYTHROPOIESIS		1 (2%)	
#MESENTERIC L. NODE	(15)	(43)	(44)
CONGESTION, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(48)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
CALCIFICATION, FOCAL			1 (2%)
CYTOPLASMIC VACUOLIZATION			1 (2%)
BASOPHILIC CYTO CHANGE		1 (2%)	1 (2%)
MEGALOCYTOSIS			1 (2%)
HYPERTROPHY, FOCAL		1 (2%)	
#LIVER/CENTRIOBULAR	(20)	(50)	(48)
METAMORPHOSIS FATTY			1 (2%)
HYPERTROPHY, NOS			1 (2%)
#LIVER/KUPFFER CELL	(20)	(50)	(48)
HYPERPLASIA, FOCAL			1 (2%)
#BILE DUCT	(20)	(50)	(48)
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
#STOMACH	(20)	(50)	(48)
INFLAMMATION, ACUTE	1 (5%)	2 (4%)	
#PEYERS PATCH	(20)	(48)	(50)
HYPERPLASIA, LYMPHOID			2 (4%)
#COLON	(19)	(49)	(49)
NEMATODIASIS		1 (2%)	
URINARY SYSTEM			
#KIDNEY/GLOMERULUS	(20)	(50)	(48)
SCLEROSIS	1 (5%)		
#URINARY BLADDER	(17)	(47)	(48)
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
HYPERPLASIA, EPITHELIAL			2 (4%)
ENDOCRINE SYSTEM			
#THYROID	(18)	(45)	(49)
GOITER COLLOID			2 (4%)
GOITER ADENOMATOUS		4 (9%)	22 (45%)
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
HYPERPLASIA, PAPILLARY		9 (20%)	12 (24%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	2 (4%)
#THYROID FOLLICLE DISTENTION HYPERTROPHY, NOS	(18)	(45) 1 (2%)	(49) 1 (2%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND CYST, NOS	(20)	(50) 2 (4%)	(50)
#TESTIS/TUBULE CYST, NOS	(19)	(50)	(49) 1 (2%)
NEPVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
63 MSAC 100 222012			CEREBRAL CORTEX THYROID UTERUS/ENDOMETRIUM

* ORGAN WITH MULTIPLE TUMORS OR MULTIPLE ORGANS WITH IDENTICAL TUMORS

DISPOSITION CODES:	ACCK: ACCIDENTALLY KILLED	NATD: NATURAL DEATH
	AUTO: AUTOLYSIS	SSAC: SCHEDULED SACRIFICE
	MISS: MISSING	TSAC: TERMINAL SACRIFICE
	MSAC: MORIBUND SACRIFICE	OTHR: OTHER

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH
4 4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*LUNG/BRONCHUS INFLAMMATION, CHRONIC	(20) 1 (5%)	(49)	(47)
*LUNG/BRONCHIOLE BRONCHIOLECTASIS	(20) 1 (5%)	(49)	(47)
*LUNG ATELECTASIS	(20) 1 (5%)	(49)	(47)
CONGESTION, ACUTE PASSIVE		1 (2%)	
PERIVASCULITIS	1 (5%)		
HEMATOPOIETIC SYSTEM			
*SPLEEN HYPERPLASIA, LYMPHOID	(19)	(49) 1 (2%)	(47) 2 (4%)
HEMATOPOIESIS			1 (2%)
*MEDIASTINAL L. NODE HYPERPLASIA, NOS	(17)	(43) 1 (2%)	(36)
*PANCREATIC L. NODE INFLAMMATION, GRANULOMATOUS	(17)	(43) 1 (2%)	(36)
HYPERPLASIA, NOS		1 (2%)	
*MESENTERIC L. NODE INFLAMMATION, ACUTE/CHRONIC	(17)	(43)	(36) 1 (3%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
HYPERPLASIA, NOS			1 (3%)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*SALIVARY GLAND INFLAMMATION, CHRONIC	(18)	(46)	(37) 1 (3%)
*LIVER	(19)	(49)	(48)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	1 (5%)		
BASOPHILIC CYTO CHANGE		4 (8%)	
EOSINOPHILIC CYTO CHANGE		2 (4%)	7 (15%)
*BILE DUCT	(19)	(49)	(48)
INFLAMMATION, CHRONIC	1 (5%)		
HYPERPLASIA, FOCAL			1 (2%)
*PANCREAS	(17)	(49)	(48)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
*STOMACH	(20)	(49)	(47)
INFLAMMATION, CHRONIC			1 (2%)
*GASTRIC MUCOSA	(20)	(49)	(47)
NECROSIS, FOCAL	1 (5%)		
*PEYERS PATCH	(20)	(48)	(44)
HYPERPLASIA, NOS		1 (2%)	
URINARY SYSTEM			
*KIDNEY	(19)	(49)	(48)
HYDRONEPHROSIS			3 (6%)
INFLAMMATION, INTERSTITIAL		1 (2%)	
PERIVASCULITIS			1 (2%)
*URINARY BLADDER	(16)	(41)	(41)
INFLAMMATION, CHRONIC		1 (2%)	7 (17%)
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
INFLAMMATION, CHRONIC FOCAL	1 (6%)	5 (12%)	2 (5%)
#U.BLADDER/MUSCULARIS INFLAMMATION, CHRONIC FOCAL	(16) 1 (6%)	(41)	(41)
ENDOCRINE SYSTEM			
#THYROID INFLAMMATION, CHRONIC FOCAL	(16)	(43)	(43)
GOITER ADENOMATOUS		25 (58%)	35 (81%)
HYPERPLASIA, PAPILLARY		7 (16%)	6 (14%)
#THYROID FOLLICLE DISTENTION	(16)	(43) 2 (5%)	(43)
REPRODUCTIVE SYSTEM			
#UTERUS HYDROMETRA	(19) 1 (5%)	(49) 3 (6%)	(47)
ANGIECTASIS		1 (2%)	
#UTERUS/ENDOMETRIUM INFLAMMATION, SUPPURATIVE	(19) 2 (11%)	(49)	(47) 7 (15%)
HYPERPLASIA, CYSTIC	5 (26%)	28 (57%)	18 (38%)
#OVARY CYST, NOS	(17) 6 (35%)	(37) 7 (19%)	(34) 2 (6%)
PAROVARIAN CYST		1 (3%)	
HEMORRHAGIC CYST			1 (3%)
ANGIECTASIS			1 (3%)
NERVOUS SYSTEM			
#CEREBRAL CORTEX PERIVASCULAR CUPPING	(20)	(48)	(41) 1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	1	1
ANIMAL MISSING/NO NECROPSY		1	2
AUTO/NECROPSY/HISTO PERF			1
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

Review of the Bioassay of 4,4'-Methylenebis(N,N-dimethyl)Benzenamine*
for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 4,4'-Methylenebis(N,N-dimethyl) Benzenamine for carcinogenicity.

The primary reviewer said that 4,4'-Methylenebis(N,N-dimethyl)benzenamine caused a dose-related incidence of follicular-cell carcinomas of the thyroid in treated rats and a significant number of hepatocellular tumors in treated mice. After briefly describing the experimental design, he said that the study was well-conducted and supported the conclusion given in the report. The primary reviewer said that 4,4'-Methylenebis(N,N-dimethyl)benzenamine should be considered as a potential carcinogen for humans.

The secondary reviewer questioned if the incidence of hepatocellular adenomas in the high dose treated male mice was statistically significant. A Program staff member noted that the response was only marginal when compared with historical control animals. The secondary reviewer suggested that it would be worth pointing out in the report summary the thyroid follicular hyperplasia observed in treated mice.

There was no objection to a recommendation that the report on the bioassay of 4,4'-Methylenebis(N,N-dimethyl)benzenamine be accepted as written.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Kenneth Wilcox, Michigan State Health Department

-
- * Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1979--281-217:3220

Library
National Institutes of Health
Bethesda, Maryland 20014

DATE DUE



**Amazing Research.
Amazing Help.**

<http://nihlibrary.nih.gov>

**10 Center Drive
Bethesda, MD 20892-1150
301-496-1080**

GAYLORD

PRINTED IN U.S.A.

NIH LIBRARY



4 0131 9373

